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The relation of male hormone to phosphatase activity in the seminal vesicle of the guinea pig[1]

by

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In honor of Professor K. Ponse on the occasion of her sixtieth birthday

Hormones are undoubtedly important enzyme regulators, although their specific mode of action is not yet clearly understood. The study of hormone-enzyme relationships has been widely undertaken, and the extensive literature is critically reviewed in the recent paper of Knox, Auerbach and Lin (1956). Studies on the male accessory reproductive glands almost invariably indicate the necessity of male hormone for normal enzyme concentration (or activity), although there are in some instances, exceptions in which the removal of the gonads has produced no change or has increased the enzyme level in some accessory glands.

The response in phosphatase activity to male hormone in the guinea pig has been investigated by Bern and Levy (1952), who found that after a long castration period of 3½ months, the enzyme activity in the seminal vesicle was greatly depressed. Their work left open the question of whether this gland could respond more rapidly to the loss of male hormone. This is especially pertinent, since it has recently been shown that other functional activities

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of this gland in the guinea pig are markedly inhibited after a much shorter castration period (LEVEY and SZEGO 1955; ORTIZ, PRICE, WILLIAMS-ASHMAN and BANKS 1956).

We are now conducting a series of biochemical and histochemical studies on the seminal vesicle of the guinea pig in order to examine the sequence of changes in metabolic activity which occur in this gland in response to hormonal alterations. This includes determinations of phosphatase activity after different castration periods and hormone treatments. The present paper is a preliminary report on the quantitative determinations of acid and alkaline phosphatase activity after several weeks of castration and subsequent treatment with male hormone.

MATERIALS AND METHODS

These experiments were performed on 25 adult male guinea pigs of a mixed strain kept in the laboratory for many years. The animals were 3 months to 1½ years of age. There were 10 normal animals in group A, 10 castrates in group B, and group C consisted of 5 castrates which had been injected with testosterone propionate.[1] The period of castration in groups B and C was 5 to 7 weeks, and the injected castrates received 2.5 mg/day of the hormone for the last 2½ weeks.

At autopsy the animals were given slight ether anaesthesia, followed by decapitation and bleeding. The paired seminal vesicles were quickly removed and weighed, immediately transferred to a dish immersed in ice, and kept very cold during subsequent manipulations. A section of the gland was slit open, the secretion carefully removed without damage to the mucosa, and the tissue washed in two changes of cold distilled water to remove the secretion completely. After the tissue was dried on hard surfaced filter paper, it was weighed and homogenized in cold distilled water. The secretion of the seminal vesicles from the normal animals was also analyzed.

Homogenates were first made at 10 mg/ml. They were further diluted to 0.5 mg/ml, after preliminary experiments indicated

[1] Grateful acknowledgment is made to Dr. Edward Henderson, of the Schering Corporation, Bloomfield, New Jersey, for his generous supply of this hormone.

extremely high enzyme activity in the tissue. The homogenates made of secretion were used at 10 mg/ml for the alkaline and diluted to 1 mg/ml for the acid phosphatases.

Phosphatase method.

The method used was that of King-Armstrong as modified by Binkley, Shank and Hoagland (1944) and by Moog (1946). Phosphatase activity was determined as μg of phenol (read from a standard curve with tyrosine) liberated by 100 mg of fresh tissue (or secretion) after incubation with disodium phenylphosphate and the appropriate buffer at pH 5.3 and 9.4. In the alkaline, $MgCl_2$ was used as activator.

Incubation time was 5 minutes for the alkaline phosphatases and 15 minutes for the acid. Each determination was made in duplicate, each tube containing 0.5 ml homogenate and 2.5 ml buffer-substrate. A third tube without homogenate served as blank. The color was read at 550 mμ in a Coleman spectrophotometer after standing for 10 to 30 minutes.

The following experiment indicated that most of the color represented enzyme activity. With tissue of one normal male, determinations were made in duplicate and one blank as usual, but one control tube was added containing homogenate and buffer-substrate, in which the reaction was stopped before incubation. Results of optical density readings showed that 85% of the color in the alkaline range and 80% in the acid, resulted from enzyme activity.

Final results on phosphatase activity were expressed as μg phenol/100 mg/minute, and they were analyzed by the Student t-test for statistical significance of the mean differences. Probability values are included in the table.

Nitrogen method.

Nitrogen was determined in the homogenates of about one-half of the animals. With the micro-Kjeldahl method 2 aliquots were run for each homogenate, with 3 standards and 1 blank of distilled water. Nitrogen units correspond to μg nitrogen per mg wet weight.

Results

The table summarizes the results obtained in the weight, phosphatase activity and nitrogen of the seminal vesicles of all the animals used. The body weight of each animal is also included, and the weight of the seminal vesicle is expressed per 1000 g body weight.

Phosphatase activity in the normal glands showed great variation which was not correlated with age or with the size of the gland. Enzyme activity in the tissue was very high, especially in the alkaline range. The secretion, however, showed extremely low levels of activity, which are not included in the table. The mean of the alkaline phosphatase in the secretion was 66, and of the acid, 100 phosphatase units. Since these levels of activity can be considered insignificant, it is indicated that the phosphatases are not normally released into the secretion.

Castration produced a very significant drop in the activity of both phosphatases ($t = 15$ and 12). Results were less variable in the castrates than in the normal animals and the variation in enzyme activity was not correlated with variation in organ weight, body weight or age.

The injection of male hormone into castrates restored the high enzyme activity and the normal weight of the gland. However, in the acid phosphatases the range of activity in the castrates and the injected castrates showed such overlap, that the difference of the two means was of doubtful significance ($P = 0.02 — 0.05$). This is very likely due to an error of sampling because of the small number of injected animals.

From the N_2 determinations it can be observed that the fresh weights of seminal vesicle tissue in the 3 groups of animals represented comparable samples. Nitrogen values were remarkably consistent in all groups.

Discussion

The results indicate that the activity of alkaline and acid phosphatases in the seminal vesicle of the guinea pig is largely under the control of male hormone and that a castration period of 5 to 7 weeks produces a marked inhibition of this activity. Thus,

the phosphatase response of this gland to male hormone can occur more rapidly than was previously reported by Bern and Levy (1952). It must still be determined whether castration for periods much shorter than 5 weeks will inhibit enzyme activity, as has been found in the rat (Stafford, Rubinstein and Meyer 1949; Porter and Melampy 1952). Levey and Szego (1955) have observed that in guinea pigs castrated for only 2 weeks the oxidative metabolism of the epithelium of the seminal vesicle is already defective. The levels of secretory activity of fructose and citric acid in this epithelium are also very low by 4 weeks of castration, and it is strongly suspected that the gland is no longer actively secreting these products (Ortiz, Price, Williams-Ashman and Banks 1956). Thus, it appears that the metabolism of this gland is affected quite rapidly by hormone deprivation.

An important point brought out in our experiments is the fact that in the normal guinea pig these phosphatases seem to be localized entirely in the tissue and are not normally released into the secretion, as are fructose and citric acid. This emphasizes for enzyme studies in this gland, the need of completely removing the secretion from the tissue by washing, as well as the desirability of performing separate analyses of the gland and of secretory material. Bern (1949) and Bern and Levy (1952), however, have stated that there was marked phosphatase activity both in the epithelium and in the secretion, but their statement was based mainly on histochemical findings, and they did not perform quantitative determinations of the secretion. Their conclusion has not been confirmed by our quantitative findings, since the secretion showed mean values of phosphatase activity which are definitely insignificant. With determinations done on cell fractions of seminal vesicle mucosa in the guinea pig, Kellerman (1955) has localized the alkaline phosphatases in the free border of the epithelium, while the acid was distributed throughout the cell. However, he did not study the secretion.

The role of the phosphatases, particularly the alkaline group, has been subject of speculation for several years. The theory of Mann and his associates (1951 *a*; *b*; 1954), further investigated by others (Parr and Warren 1951; Kellerman 1955), suggests that alkaline phosphatases facilitate fructose liberation in the seminal vesicle or other accessory glands which produce fructose. On the

other hand, WILLIAMS-ASHMAN and BANKS (1954) having observed the presence of ketose reductase in fructose-producing glands, postulated the possible role of this enzyme in the transformation of glucose into fructose with sorbitol as intermediary product. This has been confirmed by HERS (1956 *a*; *b*; 1957) who has further shown the two steps in this transformation, involving 2 different enzymes. Thus, aldose reductase facilitates the transformation of glucose into sorbitol and ketose reductase, the change of sorbitol into fructose.

It is recognized that before specific functional roles can be assigned to the alkaline and the acid phosphatases, it would be necessary to identify the different enzymes in each group. In the seminal vesicle of the guinea pig, NEWMAN, FEIGIN, WOLF and KABAT (1950) have localized by histochemical methods at least two groups of phosphatases at pH 9.2.

It can be concluded that while the functional significance of the alkaline and acid phosphatases remains unknown, there is little doubt that in the seminal vesicle of the guinea pig these enzymes are under hormonal control by the testis. Furthermore, there is a very marked regression in phosphatase activity after 5 to 7 weeks of castration.

SUMMARY

Quantitative determinations of the activity of alkaline and acid phosphatases were made in the seminal vesicle of 25 guinea pigs, including normal, castrates and castrates injected with 2.5 mg/day of testosterone propionate. Analyses were made by incubation of homogenates of washed tissue (and of secretion in the normal glands) in phenylphosphate substrate with the appropriate buffer. While phosphatase units are expressed per mg of fresh tissue, the analyses of nitrogen in tissue samples indicated no difference in protein concentration in the three groups.

In the normal tissue the activity of both phosphatases was very high, and the alkaline activity was about 4 times as high as that of the acid. The secretion of normal glands showed no significant levels of phosphatase activity.

Castration for 5 to 7 weeks caused very marked reduction in phosphatase activity in the tissue, while the injection of male hormone re-established normal levels of enzyme activity.

The effect of male hormone on seminal vesicle phosphatases in the guinea pig

Phosphatase units = μg phenol/minute liberated from phenylphosphate substrate by 100 mg wet weight of washed tissue.
Nitrogen units = μg N_2/mg wet weight.

Group	Body weight (g)	Seminal Vesicle (g/1000g body weight)	Phosphatases		Nitrogen
			Alkaline	Acid	
A. Normal	980	5.1	3323	866	
	1160	5.9	3200	826	
	568	4.6	9920	1173	27.3
	628	2.8	5760	1226	29.4
	603	3.2	2240	667	26.0
	872	4.7	4320	1147	28.0
	570	3.0	2560	1066	23.8
	480	3.3	3200	746	
	876	10.3	3280	853	
	508	4.2	4080	827	
Mean:	725	4.7	4188	940	26.9
B. Castrates	1160	1.1	740	560	
	670	0.9	720	453	31.8
	1180	2.0	560	400	—
	810	1.6	1200	960	26.0
	941	1.5	800	613	30.5
	866	1.1	720	1040	26.0
	770	1.4	800	456	26.8
	790	1.0	560	456	
	596	0.8	560	533	
	590	1.4	272	320	
Mean:	837	1.3	693	579	28.2
C. Castrates + testosterone propionate	1103	5.3	1200	613	
	768	4.3	5120	1120	25.8
	1240	4.7	4480	667	—
	770	6.0	2880	960	25.8
	982	3.1	7680	1410	29.7
Mean:	973	4.7	4272	954	27.1
Probability					
A. vs. B.	$P = .2–.3$	$P < .01$	$P < .001$	$P < .001$	—
C. vs. B.	$P = .2–.3$	$P < .001$	$P < .001$	$P = .05–.02$	—

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